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On 1 Dec. 2006

TOWNSEND and TOWNSEND and CREW LLP

Malinda Ardgit

PATENT
Attorney Docket No.: 020130-001420US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 2975
VANDER HORN, Peter B.	Examiner: Jeffrey S. Lundgren
Application No.: 10/627,592	Technology Center/Art Unit: 1639
Filed: July 25, 2003	DECLARATION UNDER 37 C.F.R. § 1.131
For: METHODS OF MAKING HYBRID PROTEINS	
Customer No.: 20350	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Peter B. Vander Horn hereby declare that:

1. I am the inventor of the subject matter claimed in the above-referenced patent application.

2. I conceived of and reduced to practice the claimed invention in the United States *prior to* November 2002. Attached Exhibits A and B provide evidence of the completion of the invention. The dates on the Exhibits and unrelated information have been redacted. All redacted dates are prior to November 2002.

3. The present invention relates, in part, to methods of generating hybrid proteins. In the methods of the invention, at least two parent protein sequences that have a common biological activity are aligned and positions at which the parent amino acid residues are different are identified (such a position is referred to herein as a "divergent site"). The codons

that encode the differing residues are compared and a nucleic acid sequence encoding a protein that is a hybrid of the parent proteins is derived. This nucleic acid sequence includes degeneracies at codons that encode divergent sites. Such a degenerate codon has at least one nucleotide position in the codon that is variable such that the codon can encode multiple parental amino acid residues at the divergent site, depending on which nucleotide is incorporated at that codon position during synthesis of a nucleic acid molecule. Libraries can thus be generated in which members are hybrids that have amino acid residues from one of the parents at some of the divergent sites and, independently, amino acid residues from a different parent at other divergent sites. The library is then screened and functional hybrid proteins are identified.

4. Prior to November 2002, I aligned a parent Pfu polymerase protein sequence and a parent Deep Vent® polymerase protein sequence and identified differences in the amino acid sequences. An *E. coli* codon usage table was used to compare the various codons that can encode the differing amino acids. I created a nuclei acid sequence that alternatively encoded differing parental amino acid residues at sites of variation in the protein sequences.

5. Oligonucleotides were designed for synthesis to assemble together to form a full length polymerase gene to make a library encoding hybrid polymerase proteins. A copy of a laboratory notebook page showing the sequences of the oligonucleotides, including the positions that can encode alternative amino acid residues at sites that differ in the parent protein sequences, is provided in Exhibit A. The oligonucleotides were synthesized and assembled by overlap extension.

6. Functional polymerase proteins encoded by members of the library were identified. An example of a PCR analysis using an exemplary hybrid polymerase protein that was isolated from the library is shown in the copy of a laboratory note book page provided as Exhibit B. Exhibit B shows a gel with the products of amplification reactions performed using a hybrid polymerase, designated "PhS1". The hybrid polymerase amplified template DNA targets of 4, 5, 9, and 13 kb in length. The gel was obtained prior to November 2002.

7. In view of the foregoing, I respectfully submit that it has been unequivocally established that the claimed invention was conceived of and reduced to practice prior to November 2002.

I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: Peter B. Vander Horn Dated: 10/30/2006

Peter B. Vander Horn

Attachments: Exhibits A and B

60893482 v1

Project code: RGT1000 Vendor: *Operon* Date: *10/18/00* Ordered by: *Yan*

	Name	Oligo Sequence (5' to 3')	Length (nt)	Scale (μmol)	Purify by	Cost($)
1	HY1o-RiNde	GGAATTCCATATGATCCTGGATGYTGACTACATCACTGAAGA	42	0.20	desalt	$35.70
2	HY1b	GGATGYTGACTACATCACTGAAGANGCCAAACCGRTTATCCGTHTSTTCAAAAAAGAGAACGGCRAATTTAAG	73	0.20	desalt	$62.05
3	HY2	AGAATCATCTTTCAGCAGAGCGTAAATGTATGGACGAAAAGKTGCGATCATRCTCAAYGTTAAATTYGCGGTTCTCTTTTTTGAA	84	0.20	desalt	$71.40
4	HY3	ATTTACGCTCTGCTGAAGATGATTCTMGATTGAKGAAGTTARAAAAATCACTGSTGAGCGCCATGGCAAGATTYTCGTATC	84	0.20	desalt	$71.40
5	HY4	GATTGGTTTGCCCAGAAATTTCTTCTYTACCTTTCCRCATCAAYGATACGAACAATCTTGCCATGGCGCTCA	73	0.20	desalt	$62.05
6	HY5	GAAGAAATTTCTGGGCARACCAATCRNGGTGTGGARACTGTATTTCGARCATCCACAAGATGTTCCGA	88	0.20	desalt	$67.80
7	HY6	AATGGAATATCGTATTCGAAGATGTCAAYAACTGCAGRATGTTCGCGAATTTTMTCGCGAATARTCGGAACATCTTYTGGATGTTCGA	88	0.20	desalt	$74.80
8	HY7	TTGACATCTTCGAATACGATATTCCATTTGCAAAGCGTTACCTCATCGACAAAGGCCTGATACCAATGGAGGCGCGAKGAAGAAC	84	0.20	desalt	$71.40
9	HY8a	CAAACTCTTCCCCTTGGTGATAGAGGGTTTTATATCCGAACGCCAGGAWCTTGATTTCTTTMTCGCCGTCCATTGGTATCA	81	0.20	desalt	$68.85
10	HY8b-BclI	ATAGCTGATCATTATAATTGGGGCTTTASCAAACTCTTNGCCTTCGTG	48	0.20	desalt	$40.80
11	HY9-BclI	ATAATGATCAGCTATGCACATGAARAMGAAGCAAAGGTGATTACTTGGAAAAAAATAGATCTCCC	65	0.20	desalt	$55.25
12	HY10	TGAGAAAGCGGTTAATCATCTCGCGCTCGGAAGATACAACCTCAACGTATGGCAGATCTATKTTTTCCAAGTAATGACC	80	0.20	desalt	$68.00
13	HY11	GCGCGACATGATTAAGCGCTTTCTCARARTTATCCGCGAGAAGGATCCGGACRTTATCRTTACTTATAACGGCGACTCTTTTGAC	85	0.20	desalt	$72.25
14	HY12	CAGTTTAATACGAGTTTTTCTGCGCGTTTCRCCAGATATGGGARGTCAAAAGAGTCGCGTTATAAGTAAYGATAA	77	0.20	desalt	$65.45
15	HY13	CGCGCAGAAAACTCGGTATTAAACTGMCTNTCGGCCGTGATGGTTCCGAGCCGAAGATGCAGCGTNTCGGCGATATGAC	80	0.20	desalt	$68.00
16	HY14	AATTACATGATACAGGTCGAAATGGATACGACCTTAAYTTCTACAGCGGTCATATCGCCGAGCACGCTGCATCTTCGG	78	0.20	desalt	$66.30
17	HY15	ATCCATTTCGACCTGTATCATGTAATTHSCGGTACTATTAACCTCCCGACTTACACTCTCGAGGCTGTATATGAAGCAATT	81	0.20	desalt	$68.85
18	HY16-AccI	GCAATCTCATCGGCGTATACCTTCTCCTTCGCTTACCAAAAATTGCTTCATATACAGCCTCGAGAGTG	69	0.20	desalt	$58.65
19	HY17-AccI	GCCGAAGGAGAAGTATACGCCSATGAGATTGCARAGCGGTGGGAAMCCGGTRAGRRCCTGGAGCGTGYTGCAAAATACTCCATG	85	0.20	desalt	$72.25
20	HY18	TTCCATTGGGARGAATTCTTTGCCGAGTTCATAAGTCRCCTTTCCATCTTCCATGGAGTATTTGCAACACGCTCGAG	78	0.20	desalt	$66.30
21	HY19	GAACTCGGCARAGAATTCTYCCCAATGGAARYTCAGCTCTCTGCCTGGTTGGCCAACCACTGTGGGATGTTYTCGTT	79	0.20	desalt	$67.15
22	HY20	GTTGCGTTCGTAGGCTTTGCGCAGGAGAWACCACTCTACGAGGTTACCGGTGGAAGAACCAGAAACATCCCACAGTGGTTGGC	83	0.20	desalt	$70.55
23	HY21	CTCCTGCGCAAAGCGTACGAACGCAACGAASTGGCTCCGAACAAGCCAKHCGAASRAGAGTATSAACGCCGTCTCCGCAGTCTT	85	0.20	desalt	$72.25
24	HY22	TTCCCAGAGGCCTTTTCTGGCTCTTTAACAWAGCCACCAGYGTAAGACTCGCGGAGACGGCGTTSATACTCT	73	0.20	desalt	$62.05
25	HY23	TAAAGAGCCAGAAAAGGGCCTCTGGGAARRCHTCGTGTTCCTCGATTTTCGCKCTCTGTATCCGTCT	67	0.20	desalt	$56.95
26	HY24-AflIII	CCGGGAGACACGTTGTGGGTAATGATAATAGACGGATACAGAGWCGCAAAATCGAGG	56	0.20	desalt	$47.60
27	HY25-AflIII	CACAACGTGTCTCCGGATACTCTCAACCKCGAGGGCTGCARARAHTATGATATTGCTCCGSAAGTAGGCCACAAGTTCTGCAAGGAC	87	0.20	desalt	$73.95
28	HY26	TCGAGCAGAYGCYCAGGAGAGACGGAATAAAGCCCGGGAWGTCCTTGCAGAACTTGTGGCCTACTT	67	0.20	desalt	$56.95
29	HY27	ATTCCGTCTCTCCTGRRGCRTCTGCTCGAKGAACGCCAARAGATTAAGNSCAAAATGAAGGWGCCHAGGATCCGATTGAAAA	83	0.20	desalt	$70.55
30	HY28	AAGAGTTTGCGAGGAKTTTAATCGCTYTTTGCGATAGTCGAGCAKTHTTTTTCAATCGGATCCTKGGMCKCCTTCATTTTG	83	0.20	desalt	$70.55
31	HY29	AGCGATTAAAMTCCTCGCAAACTCTTWTTACGGCTATTATGGCTATGCAAAAGCAGCTGGTACTGTAAGGAGTGTGCTG	80	0.20	desalt	$68.00
32	HY30	GCTCCTTCCRCACGARCTCGATGTATTYGCGACCCCAAGCAGTAAGGGACTCAGCACACTCCTTACAGTACCAGCGTGC	79	0.20	desalt	$67.15
33	HY31	AATACATGGAGTTCGTGYGGAAGGAGCTCGAAGAAAAGTTTGGCTTTAAAGTTCTCTACATTGACACTGATGGTCTCTAT	80	0.20	desalt	$68.00
34	HY32-Xba	AAATTCTAGAGCCTTTTTCTTAATTCCTCAGRCTYASCACCCGGAATAGTGCCATAGAGACCATCAGTGTCAATGTAGA	80	0.20	desalt	$68.00
35	HY33-Xba	AGGCTCTAGAATTTGTGAAATACATTAACKCGAAGCTCCCGGGTCTCCTGGAGCTCGAATATGAAGGCTTTTATRNGCGCGC	83	0.20	desalt	$70.55
36	HY34	TTTGCCTTCTTCATCAATCASCCATATYTCTTCTTGGTAACGAAGAAGCCGCGCWYATAAAAGCCTTCATATTCG	76	0.20	desalt	$64.60
37	HY35	ATATGCGSTGATTGATGAAGAAGGCAAARTTATTACTCGTGGCTCTCGAGATTGTGGGCCGTGATTGGAGCGAAATTGCGAAAG	83	0.20	desalt	$70.55
38	HY36	TTCTTCAACGTYGCCGTGTTTGAGAATAGYCTCGACAACTTTAGCTTRAGTTTCTTTCGCAATTTCGCTCCAATCACGGCG	81	0.20	desalt	$68.85
39	HY37	CTATTCTCAAACACGGCRACGTTGAAGAAGCTGTGARAATTGTAAAGAAGTAATCSAAAAGCTCHCTAAHTATGAAATTCCGCC	85	0.20	desalt	$72.25
40	HY38	CGCCTTATACTCATGCAGCGGGCGAGTAATCTGCTCATAAATCRCGAGCTTCTCTGGCCGTAATTTCATATTTAGWGAGCTTTT	83	0.20	desalt	$70.55
41	HY39	TACTCGCCCGCTGCATGAGTATAAGGCGATTGGTCCGCACGTCGTGTTGCAAAGAACTGGCTGCTARAGGCGTGAAA	79	0.20	desalt	$67.15
42	HY40-SacI	CATCGCCGCGGAGTACAATGTAGCCAATTACCATACCCGGTYTAAYTTTCRCGCCTTTAGCAGCCAGTYTCTTTGC	76	0.20	desalt	$64.60
43	HY9-16F.Bcl	ATAATGATCAGCTATGCAGATGAA	24	0.20	desalt	$20.40
44	HY9-16R.AccI	GCAATCTCATCGGCGTATACCTTCTCC	27	0.20	desalt	$22.95
45	HY17-14F.Acc	GCCGAAGGAGAAGGTATACGC	21	0.20	desalt	$17.85
46	HY17-14R.AfI	CCGGAGACACGTTGTGGGTAATG	23	0.20	desalt	$19.55
47	HY25-32F.AfI	CACAACGTGTCTCCGGATACTCTC	24	0.20	desalt	$20.40
48	HY25-32R.Xba	AAATTCTAGAGCCTTTTTCTTAA	23	0.20	desalt	$19.55
49	HY33-40F.xba	AGGCTCTAGAATTTGTGAAATAC	23	0.20	desalt	$19.55
50	HY33-40R.Sac	CATCGCCGCGGAGTACAATGTAG	23	0.20	desalt	$19.55
51	HY41-44R.Nhe	GTCATGCTAGCACTAGTGGGTACC	24	0.20	desalt	$20.40

* The price was calculated based on the special quote.

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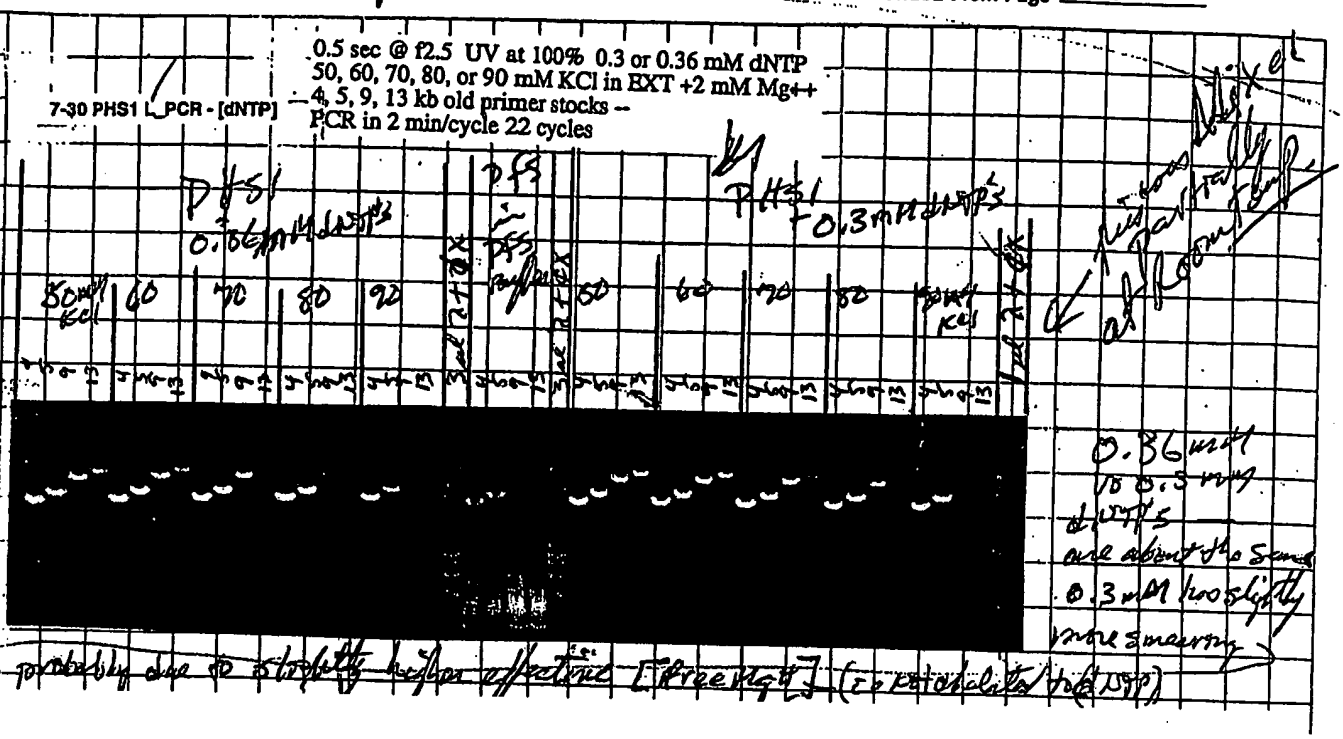
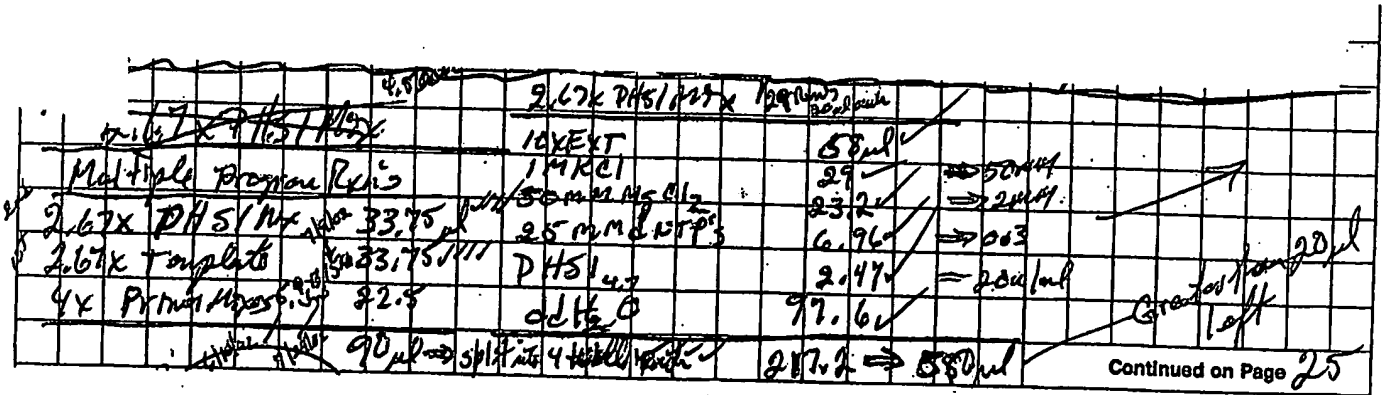
Signed Date Signed Date

7-30 PHS1 L_PCR - [dNTP]

0.5 sec @ f2.5 UV at 100% 0.3 or 0.36 mM dNTP
50, 60, 70, 80, or 90 mM KCl in EXT +2 mM Mg++
4, 5, 9, 13 kb old primer stocks --
PCR in 2 min/cycle 22 cycles





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